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PENSOFT

First records of *Hubrechtella ijimai* (Nemertea, Hubrechtiiformes) from Korea and China

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Abstract

Hubrechtella ijimai is reported for the first time from South Korea (East China Sea) and China (Yellow Sea), about 260 and 930 km from the nearest locality in Japan. Additional morphological data, confocal laser scanning microphotographs, and DNA data (COI sequences) are provided. This species possesses high intraspecific genetic COI *p*-distances for nemerteans (1.6–6.3%).

Key words

Nemertea; *Hubrechtella*; South Korea; China; COI; *p*-distances.

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Introduction

The nemertean order Hubrechtiiformes with 2 families, Hubrechtiidae (sometimes incorrectly referred to as Hibrechtidae) and Hubrechtellidae, occurs mainly on a mud or sand substrate at shallow depths of boreal, tropical, and subtropical waters. The family Hubrechtiidae is monotypic and includes the single species, *Hubrechtia desiderata* (Kennel, 1891), inhabiting the Mediterranean Sea. The family Hubrechtellidae consists of 3 genera: *Hubrechtella* Bergendal, 1902 (14 or 15 species from the Pacific, Atlantic, and Indian Oceans), *Sundbergia* Gibson, 2002 (1 species from New Zealand), and *Tetramys* Iwata, 1957 (1 species from Japan). According to Kajihara (2017), the genus *Tetramys* Iwata, 1957 is almost certainly a junior synonym of *Hubrechtella*.

Hubrechtella ijimai (Takakura, 1922) is the first hubrechtiid nemertean collected from the Pacific Ocean. The species was originally described from the Pacific coast of Honshu, Japan as *Coeia ijimai* Takakura, 1922 and was subsequently placed into the genus *Hubrechtella* (Kajihara 2006).

The present paper reports the first find of *H. ijimai* from Korea and China, identified by molecular and morphological data.

Methods

Samples were collected from 2 sites (see below) (Fig. 1). One specimen from China was fixed for morphological study in Bouin's fluid, dehydrated in 100% EtOH, cleared in xylene, and embedded in paraffin wax (m.p. 56–57 °C). Serial sections of 6–8 µm thickness were stained with Mallory's trichrome method (with the addition of chromotrope 2R). For confocal laser scanning microscopy, pieces of the body and the proboscis of the

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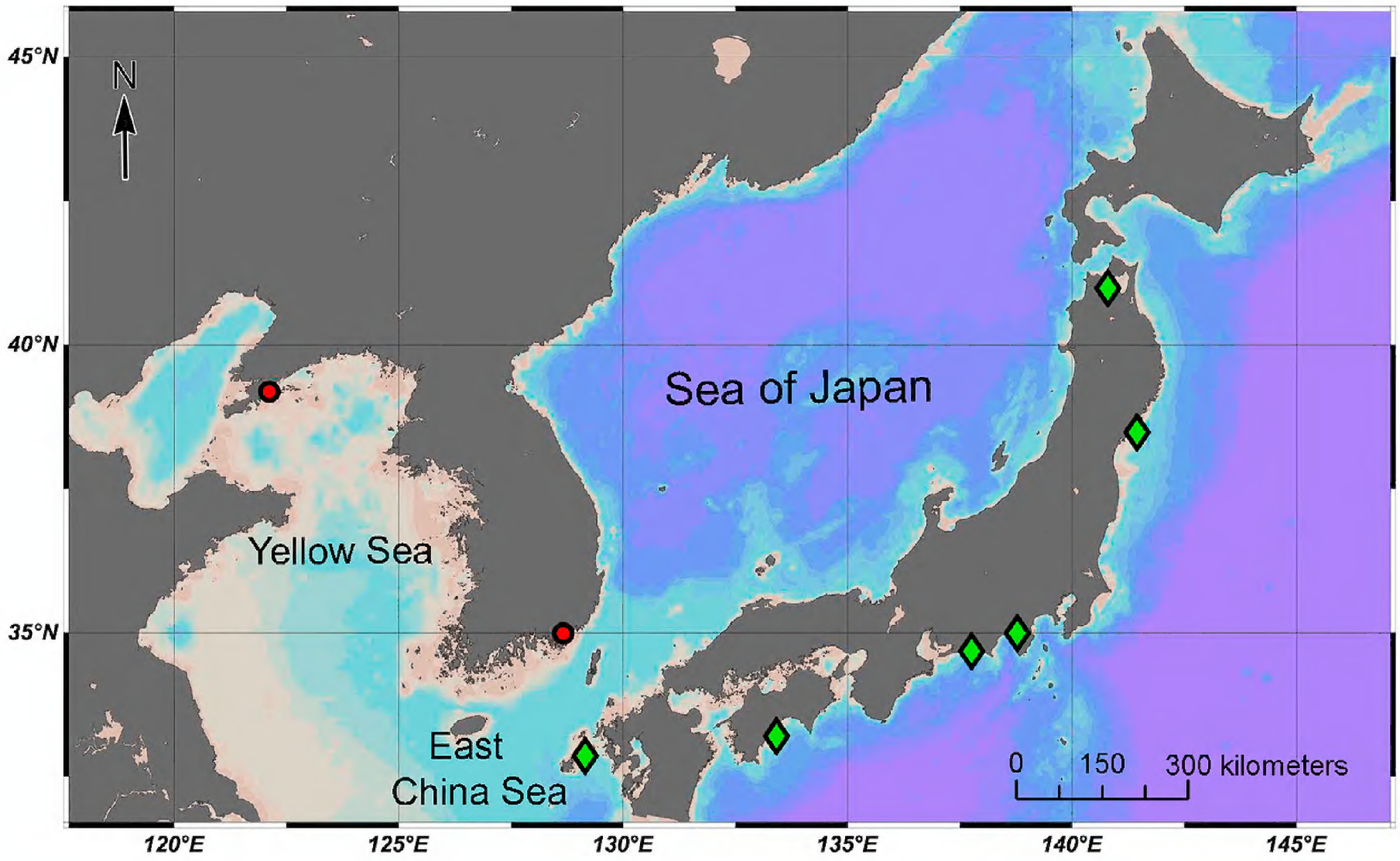

Figure 1. Distribution map for *Hubrechtella ijimai* (Takakura, 1922), with the new records in Korea and China (red circles). Green rhombs represent literature records (see Kajihara 2007).

specimen from Korea were fixed in 4% formaldehyde in phosphate-buffered saline (PBS) and stained for 10 h at room temperature with phalloidin-Alexa Fluor 633 (Invitrogen, USA) diluted (1:200) in 1% triton X-100 (Serva, German) in PBS. Pieces of the proboscis and body were immersed in Mowiol 4-88 (Sigma, USA) and mounted on glass slides. The specimens were examined under light Imager.Z2 and LSM-780 confocal microscopes (Carl Zeiss, Germany). The studied specimens are deposited in the Museum of the National Scientific Center of Marine Biology (MSCMB), Vladivostok, Russia.

Total genomic DNA was extracted from the ethanol-preserved tissues (the posterior part of the Korea specimen; the second specimen from China) using a DNA-sorb-B-100 Blood and Tissue Kit (CMD, Moscow) according to the manufacturer's protocol. The mitochondrial cytochrome *c* oxidase subunit I (COI) gene fragment was amplified using the primer pair LCO1490/HCO2198 (Folmer et al. 1994). Thermal cycling was initiated by denaturation at 94 °C for 2 min followed by 35 cycles at 94 °C for 40 s, annealing at 50 °C for 40 s, and extension at 72 °C for 1 min. After cycling, the reaction was completed with an extension phase at 72 °C for 7 min, and the reaction products were visualized in a 1% agarose gel and purified through enzymatic reaction with Exo-SAP-IT (Fermentas, Lithuania). The purified PCR products were sequenced in forward and reverse directions under conditions recommended by the manufacturer, using a BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the same primers as for PCR. DNA sequences were edited and overlapping fragments assembled into consensus sequences using ChromasPro ver. 1.34. To align the sequences, MEGA ver. 6.0 (Tamura et al. 2013) was used. The sequences were submitted to GenBank with the accession numbers # KY986686 and # KY986685. The uncorrected pairwise distances (*p*) between the COI sequences within the species were calculated in MEGA ver. 6.0. We reconstructed the haplotype networks based on the COI gene sequences using SplitsTree 4 version 4.12.3 (Huson and Bryant 2006).

Results

Order Hubrechtiiformes Chernyshev, 1995
Family Hubrechtellidae Chernyshev, 2002
Genus *Hubrechtella* Bergendal, 1902

Hubrechtella ijimai (Takakura, 1922) (Figs 1–3)
Coeia ijimai Takakura 1922: 419–422, figs.
Hubrechtella ijimai — Kajihara 2006: 28–36, figs. 15–19.

New records. 1 specimen (MSCMB 12247), South Korea: East China Sea, Jinhae Bay, north Geoje Island (34°59′34.8″ N, 128°40′24.6″ E), 12-VI-2016, low intertidal, muddy sand, coll. V.I. Radashevsky; 2 specimens (one is in the form of slides of transversal histological sections, MSCMB 12248; the second was used for sequencing COI); China: Yellow Sea, Dengshahe, Dalian, Liaoning (39°11′ N, 122°07′ E), 29-VII-2006, intertidal, sandy sediment contained a little organic matter, coll. S.-C. Sun.

Sequences. Two COI sequences were deposited in GenBank (accession # KY986686 for the specimen from

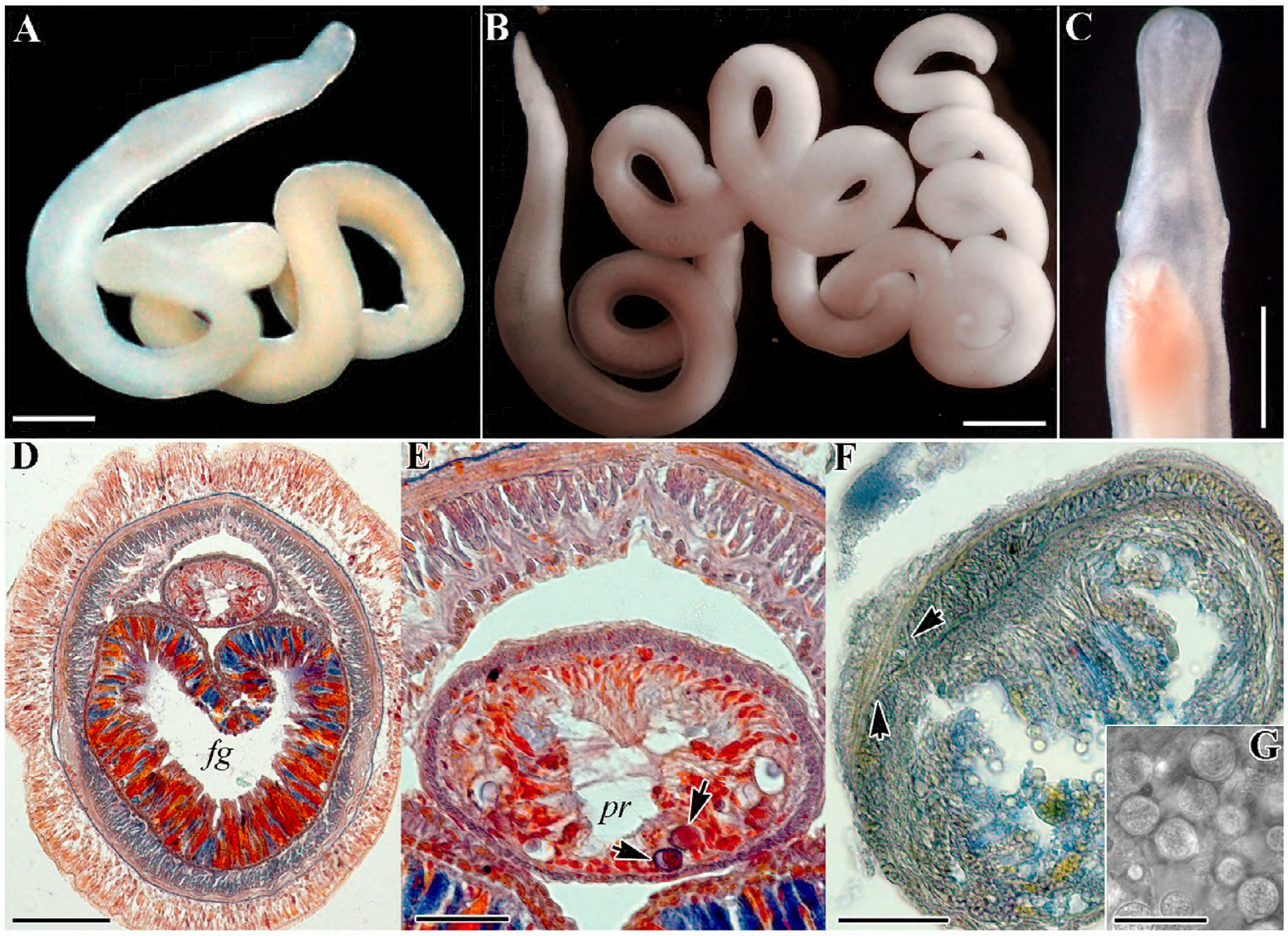

Figure 2. *Hubrechtella ijimai* (Takakura, 1922) from Korea (**A, G**) and China (**B–F**). **A, B.** Living specimens. **C.** Anterior end of the body. **D.** Transverse section of the foregut region. **E.** Transversal section of the proboscis and rhynchocoel (arrows indicate spherical pseudocnidae). **F.** Transversal section of the proboscis (arrows indicate muscle cross). **G.** Spherical pseudocnidae (*in vivo*). Abbreviations: fg, foregut; pr, proboscis. Scale bars: A–C = 1 mm; D = 100 µm; E–G = 20 µm.

Korea and # KY986685 for the specimen from China).

Type locality. Enoura Bay (Shizuoka Prefecture) and Tateyama Bay (Chiba Prefecture), Honshu, Japan (Takakura 1922).

Previous records. Japanese islands (Shizuoka, Chiba, Aomori, Miyagi, Kôchi, Fukue, and Nagasaki Prefectures) (see Kajihara 2007) (Fig. 1).

Diagnosis. This species can be distinguished from the other species of the genus *Hubrechtella* by the following combination of characters: body 3–20 cm long, translucent, whitish, spirally coiling when contracted, with caudal cirrus. Head rounded and wider than neck foregut. Lacunar network present, U-shaped blood lacuna absent in foregut region, mid-dorsal blood vessel penetrates rhynchocoel wall. Longitudinal body wall musculature lacks zigzag muscles. Proboscis with muscle cross, proboscis epithelium with "spherical bodies".

Comments. The so-called "spherical bodies" in the proboscis epithelium of *H. ijimai* are spherical pseudocnidae 10–13 µm in diameter and with central filament (Figs 2E, G). The same pseudocnidae were described in *H. atypica* Senz, 1992, *H. globocystica* Senz, 1993, and *H. kimuraorum* Kajihara, 2006 (see Kajihara 2006). Confocal laser scanning microscopy revealed several new characters for *H. ijimai*: crisscrossed diagonal muscles are present between the outer circular and the inner longitudinal body musculature in the anterior part of the body (Fig. 3B); the muscle "cross" is present between the rhynchocoel musculature and the circular body wall muscle layer (Figs 3E); the foregut musculature consists of inner circular and interwoven outer longitudinal muscles (Fig. 3F); isolated bundles of circular muscles have been found outside the brain (Fig. 3C); the proboscis musculature consists of 4 layers, as described for *Hubrechtella juliae* Chernyshev, 2003 (Chernyshev et al. 2013), with one irregular muscle cross, but the weakly developed second muscle cross is also sometime visible (Fig. 3D).

Discussion

According to our morphological observations, the present specimens are identified as *H. ijimai*. Like most *Hubrechtella* species, *H. kimuraorum* Kajihara, 2006 can be distinguished from *H. ijimai* by the lack of the muscle cross in the proboscis, by the spacious U-shaped blood lacunar connection below the foregut, and the shape of the head, which is narrower than the trunk in *H. kimuraorum* (Kajihara 2006). Unfortunately, only 18S

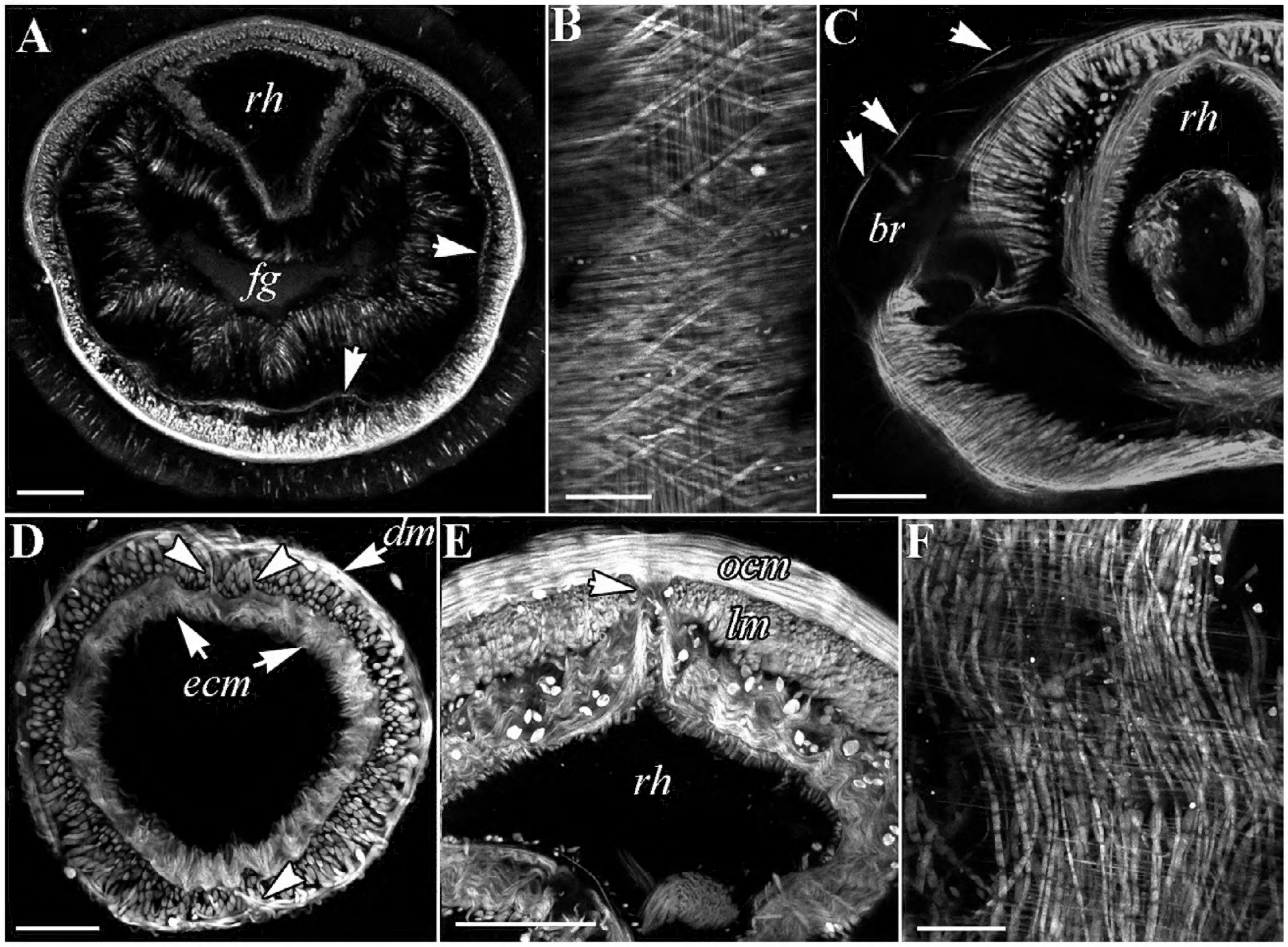

Figure 3. CLSM micrographs (Z-projection) of *Hubrechtella ijimai* (Takakura, 1922) from Korea. **A.** Transversal section of the body in foregut region (arrows indicate foregut musculature). **B.** Longitudinal section of the body wall musculature. **C.** Transversal section of the body in brain region (arrow indicate circular muscles outside the brain). **D.** Transversal section of the proboscis (arrowheads indicate muscle crosses). **E.** Transversal section of the rhynchocoel and body wall musculature (arrow indicates "cross" between body wall circular musculature and rhynchocoel). **F.** Longitudinal section of the foregut musculature. Abbreviations: br, brain; dm, outer diagonal musculature; ecm, endothelial circular musculature; fg, foregut; lm, longitudinal musculature; ocm, outer circular musculature; rh, rhynchocoel. Scale bars: A, C, E = 100 µm; B, D, F = 50 µm.

DNA sequence is available for *H. kimuraorum*, and this sequence is identical to that of *H. ijimai*. The presence of the muscle cross in the proboscis of *H. ijimai* is a unique feature among hubrechtiid nemerteans. A confocal laser scanning microscopy observation showed that the proboscis in *H. juliae* possesses "internal muscle crosses" (the crisscrossed zone in the outer diagonal musculature), which are not visible in histological sections (Chernyshev el al. 2013). The muscle cross in *H. ijima* is similar to that in most heteronemerteans, but it has an irregular structure and consists of a few irregular diagonal muscle strands. We detected not only a dorsal but also a low observable ventral cross.

The uncorrected pairwise distances between the mitochondrial COI sequences of the specimens from Korea and China and that of 2 specimens of *H. ijimai* (Kvist et al. 2014, Sundberg et al. 2016) obtained from Japan (GenBank Accession # KF935520 and KU840155) were from 1.6 to 5.6% (Table 1). The largest *p*-distance (6.3%) was observed between the samples from Korea and China. It should be noted that the genus *Hubrechtella* has 2 sibling species, *H. dubia* and *H. juliae*, with COI *p*-distances of 6.0–7.5% (mean: 6.6%), but the intraspecific *p*-distances among 26 samples of *H. dubia* are only 0.0–1.2% (mean: 0.4%). We calculated these *p*-distance values using GenBank COI sequences. At the same time, the *p*-distances among three specimens of *H. ijimai* (from Japan and Korea) are 1.6 and 2.2%, that is, the *p*-distances among sequences of *H. ijimai* do not show any clues of potential gaps. The Korean and Chinese samples have a higher distance but both are included in one haplotype network (Fig. 4) and have a very similar internal morphology. The specimen from China is distinguished by the short caudal cirrus, which is long in *H. ijimai* (Kajihara 2006), but the caudal cirrus could be damaged. Therefore we are confident that the specimen from China can be referred to as *H. ijimai*

Based on the literature data, the geographical distribution of *H. ijimai* seems to be limited to the coasts of Japan (from Fukue to Asamushi), Korea, and China. This

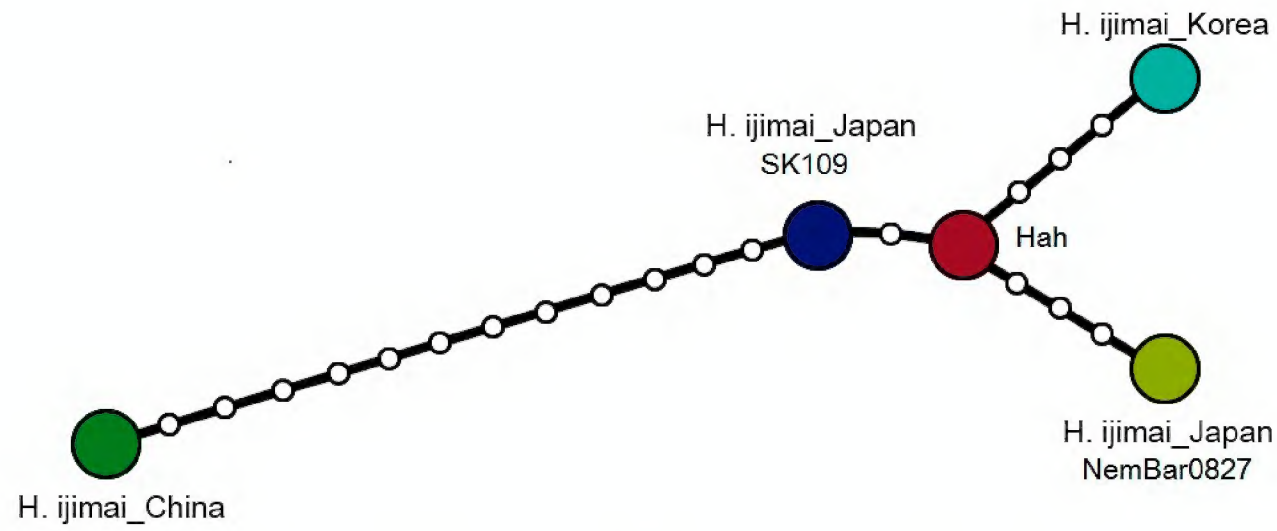

Figure 4. The haplotype network based on COI for 4 *Hubrechtella ijimai* specimens. Hah, hypothetical ancestral haplotype; the number of blank circles corresponds to the number of nucleotide substitutions between the haplotypes.

Table 1. Pairwise uncorrected *p*-distances between *Hubrechtella ijimai* specimens for COI

	***H. ijimai*_Korea**	***H. ijimai*_China**	***H. ijimai* NemBar0827**	***H. ijimai* SK109**
***H. ijimai*_Korea**	—	6.3	2.4	1.6
***H. ijimai*_China**	—	5.6	4.8	
***H. ijimai* NemBar0827**		—	1.6	
***H. ijimai* SK109**			—	

species has not been found in Peter the Great Bay (the best studied bay in the Sea of Japan), where the other species, *H. juliae*, is very common (Chernyshev 2014).

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Authors' Contributions

AC identified the species, performed the microscopic work, and wrote the text; SCS collected specimens, prepared the macroscopic data, and wrote the text; NP and CYS made DNA analysis.

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